



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of : Christian Caspersen
Serial No. : 09/806,457
Filed : 14 June, 2001
Title : An apparatus for determining the position of an object

Examiner: Lee, Shun K
Art Unit: 2884
Attorney's docket: 2836-0153PUS1

DECLARATION by Preben Buchhave, under 37 C.F.R. § 1.132

I, Preben Buchhave, declare and state as follows:

1. I am employed as a Professor in the Department of Physics at the Technical University of Denmark. for brief CV, see Appendix 1.
2. I have studied the above-mentioned application (hereinafter referred as "present invention"). In addition, I have reviewed the Final Office Action of 11 March, 2009 and have also evaluated Malin et al. (US 5,377,002) for the purpose of measuring fluorescence from stained microscopic biological objects, as described in the present invention.

3. The Office Action, in Pages 6 and 7, asserts that

"The apparatus of Malin et al. lacks to filter through the beam-splitter fluorescent light emitted from the specimen, thereby allowing fluorescent light from fluorescently marked objects to pass through the beam-splitter to the detector.... Hamashima et al. teach to provide a dichroic mirror for simultaneously detecting and the fluorescence ... from the pattern.... Therefore, it would have been obvious to provide a dichroic mirror as the at least one beam-splitter and other optical components in the apparatus of Malin et al. in order to obtain..... fluorescence measurements at a desired resolution..."

4. The measurement problem in the present invention is to identify among a large background of uninteresting specimen a few objects of interest, e.g. cells of interest in medical diagnostics. The objects of interest have been attached to a substrate (solid support) and dyed with a fluorescent marker. The technical problem is to scan the substrate and to detect the fluorescence signal from the objects of interest. When an interesting object is recognized, its location on the substrate is stored in a computer system to allow this specimen to undergo subsequent closer inspection. As the object is of microscopic size, the fluorescence signal is necessarily very weak, and the apparatus needs to be optimized for collecting the weak fluorescence signal.
5. Fluorescence is generally randomly scattered in all directions. This general tendency may be modified by specific conditions such a preferred orientation of the fluorescent markers or by re-absorption and secondary scattering in the specimen itself. In general, however, a large collection aperture without obstructing elements is essential.
6. An optimized size and shape of the illumination of the specimen, specifically designed for the purpose of detecting the fluorescence form the specimen at hand, for example, a biological cell sample fixed on a plane substrate and dyed with a fluorescent marker, is an essential requirement for the functioning of the apparatus.
7. With careful regard to the omnidirectional scattering of fluorescent radiation, weakness of the fluorescence from a microscopic object and the need for recognition of the object of interest; unwanted light must be removed as efficiently as possible while fluorescent light is obstructed as little as possible. This may be achieved in different ways, but essential ingredients include low loss, efficient dichroic beamsplitters or filters without material obstructions (holders or mounting rings), which could reduce the collection of fluorescence.
8. An essential part of Malin et al. system is the use of a dark field stop to block light scattered directly back into the receiver optics. Such directly back scattered light is detrimental to the functioning of the Malin et al.'s system, whose purpose is detection of light diffracted at large angles from the surface defects.
9. The principle of the present invention is to collect as much fluorescent light as possible from the sample, including directly and backscattered light.

10. The study outlined below was conducted and it demonstrates that fluorescence from a sample cannot be detected in a dark field stop based microscopic assembly of Malin et al.

11. Study description:

The aim of the study was to test whether fluorescence can be detected in a dark field microscopy of Malin et al. and whether inclusion of a dichroic mirror, as proposed in the Office Action, is possible in the set up of Malin et al.

Experimental Approach:

Malin et al. system is configured according to the following figure. This set up is a modification of a fluorescence microscope and emulates the principle described in Malin et al. The system combines illumination and light collection on one side of the substrate.

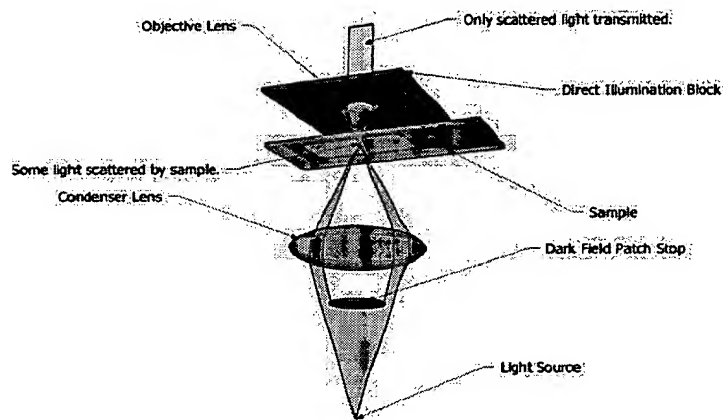


Fig. 1: Diagram illustrating the light path through a dark field microscope

A ratio of 16/24 (dark stop/ condenser lens) is used in the dark field aperture. A number of measurements are performed with and without the dark field stop, the results of which are included below.

Results & Evaluation:

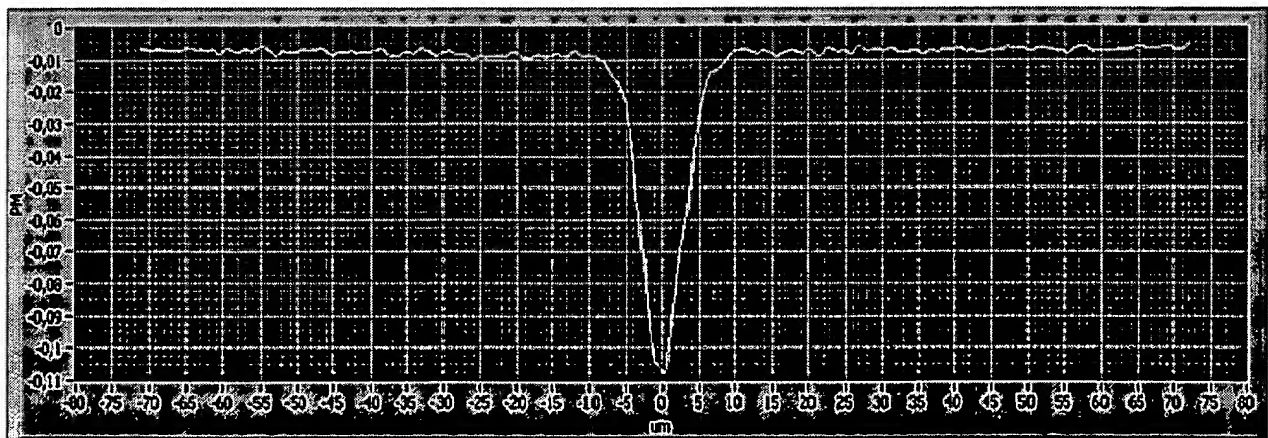


Fig. 2: Characteristic signal from the cell in Malin et al. apparatus

The signal is voltage from the photo detector (Photomultiplier tube) vs. Position on the disc. Signal level typically varied between -0.1V and -0.5 V.

The noise includes shot noise from the Photomultiplier tube (spontaneous emission), and electrical noise and quantization noise from the A/D converter. The noise level is typically 0.01 V. This noise level is present with complete darkness on the photo detector.

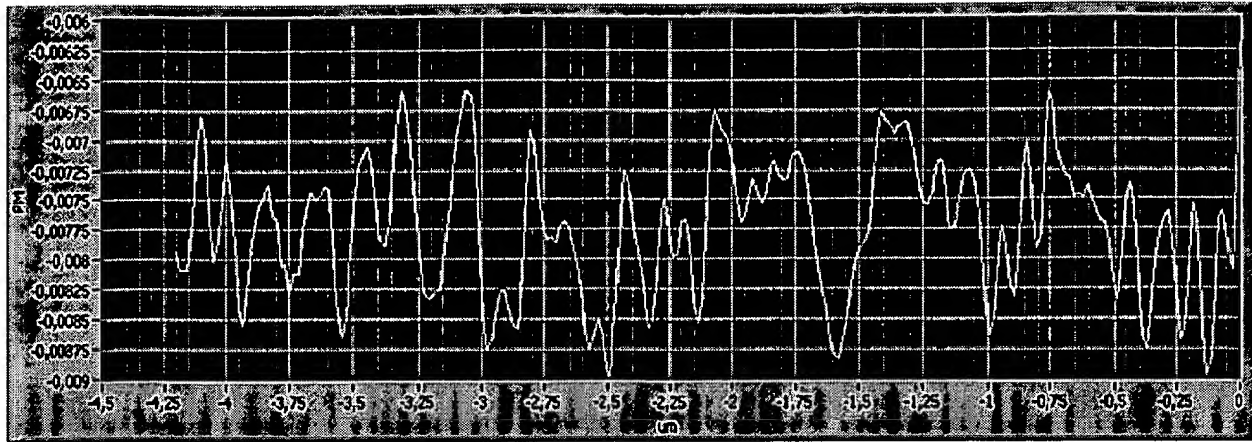


Fig. 3: Characteristic noise level in Malin et al. system

In this configuration, the signal at the detector from sample targets is at a level of 0.1 V. The noise level is at 0.01 V, thereby providing a 10 dB Signal/Noise (S/N) characteristic for these cells. This shows that a dark field system is not relevant and will not lead to any useful result.

The subsequent measurements present the distribution of signals from individual samples, as a histogram of sample voltages, recorded during a scan with and without a dark field stop. It is important for the efficient functioning of the present invention that the histogram shows a significant number of high voltage signals, which indicate an object of interest with fluorescence of the right wavelength having passed through the dichroic filter. The measurements compare the unobstructed light collection to situations where the path is blocked by a dark field aperture stop. The measurements are taken with the same settings of instrument parameters, such as detector high voltage and amplifier gain.

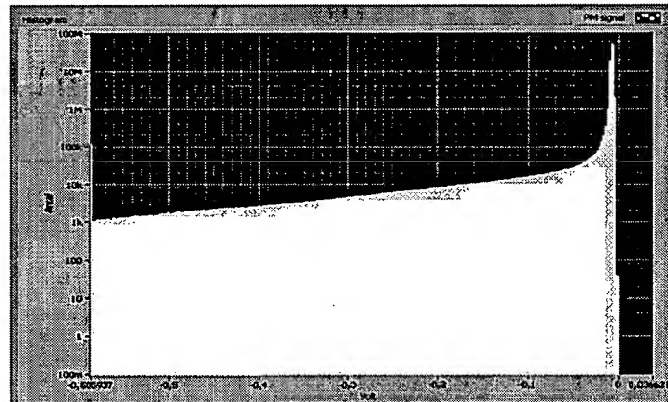


Fig. 4: Distribution of signal intensities in the Present Invention implementation

The graph in Fig. 4 represents the number of signals recorded as a function of the signal voltage. 0V is on the right hand side and maximum voltage (-0,5V) on the left hand side. The distribution is for each recorded sample.

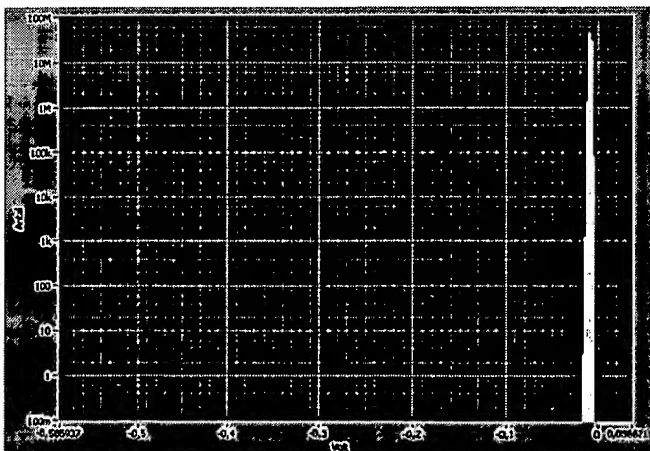


Fig. 5: Distribution of signals in the dark field configuration of Malin et al.

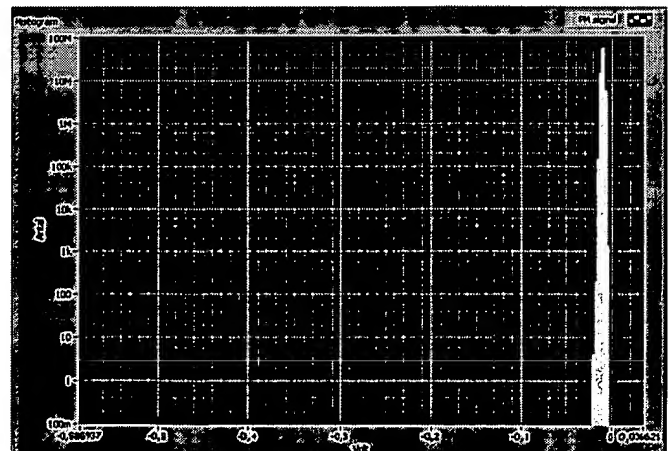


Fig. 6 Distribution of signals with a closed shutter

With the dark field configuration of Malin et al., as shown in Fig. 5, all samples are around 0 V. This means that an insufficient signal reaches the photo detector. The above graph is a distribution of signals from the entire disc. The histograms for arrangements with aperture stops show a complete lack of high voltage samples. An attempt to obtain signals using a greater collection aperture ($NA=0.6$ instead of $NA=0.7$) failed. This distribution is comparable to a system with a completely closed shutter, as represented in Fig. 6. The noise-level is approximately 10dB lower than the signal level.

These experiments demonstrate clearly, that a dark field configuration is incapable of detecting fluorescent signals. Use of dark field configuration is an important and necessary element in Malin et. al. There is no teaching in Malin et al. directed towards the present invention and therefore, Malin et al. provides no basis to formulate the present invention.

12. The measurements reliably present the difference between the apparatuses of the present invention and Malin et al. and also illustrate the unsuitability of any system, which contains an obstruction in the fluorescence collection such as a dark field aperture stop or any other object blocking the fluorescence reception.
13. In order to achieve a fine resolution when scanning of the surface, the incident illuminating beam must necessarily have a relatively small numerical aperture, which unavoidably results in an obstruction of fluorescent light from the sample.
14. The inclusion of dichroic mirror in Malin et al.'s apparatus will not overcome the limitation introduced by the dark field stop and therefore no fluorescence based meaningful result can be obtained unless the dark field stop is removed from Malin's apparatus. However, as already stated, the apparatus of Malin et al. relies on using a dark field configuration, using a dark field stop to block light scattered directly back into the receiver optics and to detect light diffracted at large angles from the surface defects.
15. In conclusion, the apparatus of Malin et al. is neither capable of measuring the fluorescence from microscopic samples because it requires blocking of backscattered light with a dark field aperture stop nor there exists any provision of including a dichroic mirror in the apparatus without disrupting the working principle of the Malin et al.
16. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: June 20, 2009

Signature:



Appendix 1: Brief CV for Preben Buchhave

I am employed as a Professor in the Department of Physics at the Technical University of Denmark. I have a MS in atomic physics from the Technical University of Denmark and a Ph.D. from SUNY Buffalo. I have worked at the Westinghouse R&D Center, Pittsburgh, and I have been manager of Research and Development at Dantec MT, Skovlunde, Denmark and Vice President of Dantec Research Equipment Inc., New Jersey. I have managed many R&D projects and systems development projects for universities, Government Institutions and universities worldwide and in particular in the US. Since 1988 I have been a full professor at DTU, Dept. of Physics, working with optical measurements, nonlinear optics and quantum optics.